

N.A.S. SYMPOSIUM: PLANT GROWTH REGULATION

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Plant Productivity and the Control of Photorespiration

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The Green Revolution of the last decade has seen the yield of some food crops at least doubled by genetic alterations of plant stature and the ability of plants to respond to increased fertilizer. Since only 5-10% of the dry weight of plants comes from minerals and nitrogen in the soil, it is becoming more difficult to obtain further increases in productivity by this approach. Even scientists associated with the Green Revolution believe they have reached a plateau by these methods (1). Therefore, the next large increases in productivity must come from increasing the 90-95% of the dry weight that comes from the assimilation of airborne CO₂ during photosynthesis.

The productivity of plants (dry weight per unit of ground area) is determined by the gross CO₂ assimilation during photosynthesis minus the CO₂ released during respiration. The "dark" respiration processes of green plants, which probably also occur as well in the light, are biochemically similar to those found in animal tissues and many microorganisms, and are essential for the growth and maintenance of plant cells. It is not certain whether all of the dark respiration is essential, or whether some portion of it may sometimes be uncoupled from ATP production. This wasteful portion of respiration could cause diminished productivity (2). However, it is well documented that many plant species and varieties respire away large portions of their recently fixed CO₂ during illumination by an entirely different biochemical process of respiration, known as photorespiration. Since photorespiration is often much faster in terms of CO₂ production than dark respiration, photorespiration greatly lowers plant productivity where this occurs. Some biochemical and plant breeding experiments will be described that hold promise for retention of much of the photorespired CO₂, leading to large increases in crop yields.

Relation of net photosynthesis to productivity among species

Crop species vary greatly in their yields of dry weight. For example, from statistics of average market yield in the United States (Table 1), one can calculate that the crop growth rate for maize silage, sorghum silage, and sugarcane (cane) is at least double that for spinach, tobacco (leaf plus

stalk), and hay grasses (3). The higher-yielding leafy species all have low fluxes of photorespiration compared with the less efficient species.

Table 2 contains typical values of CO₂ assimilation taken from the literature; it shows that much faster rates are usually found in the higher-yielding tropical grasses and in some weeds, as compared with many common crop plants—including spinach, tobacco, and orchard grass—that are lower yielding. A large part of the differences in net photosynthesis between the efficient and nonefficient species can be explained by the much slower rate of photorespiration that is encountered naturally only in the efficient plants.

Characteristics of photorespiration

Since photorespiration is such an important process, it is interesting to speculate on why its discovery was so long delayed. Earlier attempts to detect it failed largely because experiments were unwittingly performed under conditions that tended to eliminate or obscure it—high concentrations of CO₂, low light intensities, or low concentrations of O₂. Photorespiration occurs by a biochemical mechanism very different from dark respiration, since the primary substrate glycolate is produced rapidly in inefficient photosynthetic species only under the opposite conditions of those just mentioned. Moreover, the absolute measurement of photorespiration (loss of fixed CO₂) is difficult because it must be detected while the main flux of CO₂ is towards assimilation in the leaf chloroplasts. Hence, the assay of photorespiration by any procedure that requires an analysis of the ambient atmosphere must underestimate it to some degree because of the competition for the released CO₂ by the chloroplasts. Nevertheless, rates of photorespiration of more than 50% of net photosynthesis are often encountered in leaves, or at least 100 μmol of CO₂ are evolved per mg of chlorophyll per hr (13 mg of CO₂ dm⁻² hr⁻¹).

"Photorespiration" was first used in its present sense by Decker and Tió in 1959 (4) to explain the CO₂ outburst after illumination shown by leaves of many species that results as an aftermath of photorespiration. About this time, studies on the inhibition of glycolate oxidation *in vivo* also showed that a large part of the CO₂ assimilated during photosynthesis could be normally metabolized through glycolate, and sug-

Abbreviations: INH, isonicotinic acid hydrazide (isoniazid); α-HPMS, α-hydroxy-2-pyridinemethanesulfonic acid.

TABLE 1. Average yields of several leafy crops* (from ref. 3)

Crop	Calculated crop growth rate (g dry wt per m ² ground area per week)
Maize silage	47
Sorghum silage	43
Sugarcane (cane)	50
Spinach	13
Tobacco (leaf plus stalk)	25
Hay	20

* Initial average market data obtained from *Agricultural Statistics*, 1969, U.S. Department of Agriculture.

gested that this respiratory system must be important during photosynthesis (5, 6). By the early 1960s it was realized that some crop species had much higher rates of net photosynthesis than many other species (Table 2). In 1964, I pointed out that the properties of photorespiration and the characteristics of the synthesis and oxidation of glycolate had much in common (7). Subsequent work has confirmed the importance of glycolate as the primary substrate of photorespiration.

There are, as in all newer subjects that arouse considerable interest, some controversial aspects about the interpretation of experiments relating to photorespiration. Readers wishing to familiarize themselves with other points of view are advised to consult reviews relating to this subject written by Jackson and Volk (8), Goldsworthy (9), Tolbert (10), Hatch and Slack (11), Black (12), Walker and Crofts (13), and Gibbs (14). A book consisting of papers mostly concerned with C₄ metabolism (efficient photosynthetic species) has been published with excellent assessments after each section (15), and my views are expressed in greater detail in a book largely concerned with photorespiration and its relation to plant productivity (3).

The ¹⁴C assay of photorespiration

Photorespiration may be detected and assayed in several different ways; each method has certain advantages and pitfalls associated with it (3). An assay of photorespiration used

TABLE 2. Typical rates of net photosynthesis in single leaves of various species at high illuminance, 300 ppm of CO₂, in air at 25°–30° (from ref. 3)

Species	Net photosynthesis (mg of CO ₂ dm ⁻² hr ⁻¹)
Maize	46–63
Sugarcane	42–49
Sorghum	55
Pigweed, <i>Amaranthus edulis</i>	58
Bermuda grass, <i>Cyndon dactylon</i>	35–43
Spinach	16
Tobacco	16–21
Wheat	17–31
Rice	12–30
Orchard grass	13–24
Bean	12–17

most often in my experiments (16, 17) consists of labeling leaf disks under constant illumination with ¹⁴CO₂ after the disks have had a preliminary period in normal air at 30°–35°. The ¹⁴CO₂ is first released into a closed system under conditions where the ¹⁴CO₂ is completely fixed in about 15 min. The system, however, remains closed for 45 min to allow steady-state conditions to prevail. Then, at zero time, CO₂-free air is rapidly swept over the leaf disks (at least three flask volumes per min) and the ¹⁴CO₂ released from the tissue is collected and measured.

The CO₂-free air is used in the assay to change the main flux of CO₂ from the normal uptake during photosynthesis to a greater release of photorespiratory CO₂ to the atmosphere. Independent experiments have established that photorespiration is fairly constant between the range of "zero" CO₂ and 300 ppm (normal concentration) in the ambient atmosphere (18, 19). If a shorter time than 45 min is used for the period of ¹⁴CO₂ fixation in the assay, the light to dark ratio (see below) is little affected (17).

After a period in the light, the ¹⁴CO₂ released in a subsequent period of darkness is also measured. This procedure permits a calculation of the ratio of the ¹⁴CO₂ released during a fixed time, usually 30 min, after a constant rate is achieved in the light and dark (the light/dark ratio). Fig. 1 shows a typical assay performed with a standard tobacco variety and with hybrid maize. The light/dark ratio for tobacco is usually between 3 and 5, indicating that photorespiration exceeds dark respiration by about 3- to 5-fold. By comparison, this ratio for maize is usually less than 0.1, showing that this species has a very much slower rate of photorespiration than tobacco.

Glycolate biosynthesis and photorespiration

It has already been indicated that the biosynthesis and further metabolism of glycolate have many characteristics in common with those of photorespiration; this similarity suggested that photorespiratory CO₂ might be derived from the breakdown of glycolate. Direct evidence for the role of glycolate in photorespiration comes from experiments on conditions for ¹⁴CO₂ release by leaf tissue supplied with ¹⁴C-labeled glycolate, and by use of metabolic inhibitors of the oxidation of glycolate *in vivo*.

Although glycolic acid is a simple two-carbon compound,

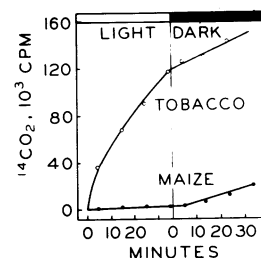


FIG. 1. Comparison of ¹⁴CO₂ released in the light (photorespiration) and dark by tobacco and maize disks at 30°. The ratio of the ¹⁴CO₂ released light/dark is 3.2 for tobacco and is less than 0.1 for maize. The differences lie in the photorespiration, since the rates of dark respiration are essentially the same. After 45 min in air in 21,400 lux of illumination, the flasks were closed and ¹⁴CO₂ (1.08 × 10⁶ cpm) was released. After another 45 min, at zero time, CO₂-free air was passed through the vessels at three flask volumes per min. The released ¹⁴CO₂ was collected and the radioactivity was determined (17).

$\text{CH}_2\text{OH}-\text{COOH}$, there is still uncertainty about the mechanism of its biosynthesis in photosynthetic tissues. It is well established that rapid synthesis occurs in light (probably in the chloroplasts), and in the relatively low concentrations of CO_2 and high concentrations of oxygen normally found in the atmosphere. These are characteristics consistent with its role as the primary substrate of photorespiration.

Several biochemical mechanisms for glycolate biosynthesis have been proposed (3); all of them have some merit and plausibility, although none can account for all of the known properties of the biosynthetic pathway that produces this substance. The older proposals suggest that glycolate arises from a two-carbon fragment derived from the Calvin photosynthetic carbon reduction cycle, that it is produced from a novel carboxylation reaction, or that it is synthesized by the reduction of glyoxylate. The major obstacle to agreement on the importance of any one mechanism is that none of the reconstructed biochemical systems can synthesize glycolate at rates anywhere near those required of a substrate of photorespiration; that is, at rates that are 50% or more of net photosynthesis.

Most recently, Ogren and his colleagues (20, 21) have discovered that phosphoglycolate is synthesized from ribulose diphosphate in the presence of purified ribulose diphosphate carboxylase in an atmosphere of O_2 . Since an active phosphoglycolate phosphatase is present in leaves (22), this reaction would provide a reasonable pathway for glycolate biosynthesis. However, under optimal conditions (100% O_2), the rate of phosphoglycolate formation was only 15% of the rate of CO_2 fixation under optimal conditions by the enzyme (23). Hence, these rates also appear to be too slow to account for the rate of glycolate biosynthesis needed for photorespiration.

There is no reason for believing that CO_2 is fixed by only one carboxylation reaction in any photosynthetic system (3). Neither is there any reason for believing that one pathway of glycolate synthesis is dominant in all tissues. Experiments with labeled precursors have shown that multiple pathways of glycolate synthesis exist simultaneously in *Chlorella* (24, 25)

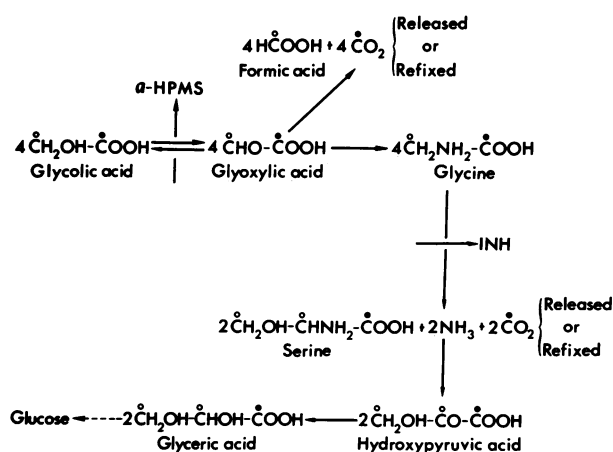


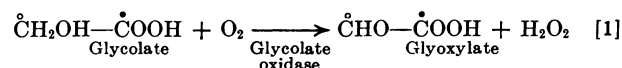
FIG. 2. Schematic diagram of the glycolate pathway of carbohydrate synthesis, showing the likely sources of photorespiratory CO_2 . (●) from C-1 of glycolate; (○) from C-2 of glycolate. α -Hydroxy-2-pyridinemethanesulfonic acid (α -HPMS) is shown blocking the oxidation of glycolate to glyoxylate. This inhibitor causes glycolate to accumulate. Isonicotinic acid hydrazide (INH) inhibits the conversion of glycine to serine, and results in glycine accumulation.

and also in higher plants (26). Glycolate is synthesized rapidly in light from $^{14}\text{CO}_2$ in leaves with high rates of photorespiration, such as tobacco (and the carbon atoms of the glycolate produced have a specific radioactivity similar to that of the $^{14}\text{CO}_2$ supplied). It is synthesized slowly—but more readily from organic acids (pyruvate, phosphoenolpyruvate)—in leaves with slow rates of photorespiration such as maize, as compared with tobacco (26).

Specific inhibitors of glycolate synthesis that would not inhibit CO_2 assimilation during photosynthesis would be most useful for study of the mechanism of biosynthesis, as well as for an evaluation of glycolate metabolism in photorespiration. Such inhibitors are being sought. Even though the biosynthetic pathway is uncertain, it is possible to assay for potential inhibitors of glycolate biosynthesis by floating leaf disks on solutions of the compound to be tested and then measuring the initial rate of glycolate accumulation after adding an effective inhibitor of glycolate oxidase. In this way, I found that isonicotinic acid hydrazide (INH), which Goldsworthy (16) had shown to be an inhibitor of photorespiration, slows the rate of glycolate synthesis under conditions similar to those used to inhibit photorespiration (26).

Glycolate oxidation and photorespiration

Since glycolate is synthesized rapidly by leaves with high rates of photorespiration (see below), yet is normally found in low concentrations (less than $0.4 \mu\text{mol/g}$ of fresh weight), it must turn over very rapidly. The oxidation of glycolate in leaves is catalyzed by an active enzyme, glycolate oxidase, which was first described by Kolesnikov (27) and Clagett *et al.* (28). The enzyme is a flavoprotein (29, 30) that catalyzes the reaction:



In the absence of catalase, the glyoxylate formed is oxidized nonenzymatically by the H_2O_2 to produce formate and CO_2 , the CO_2 arising from the carboxyl-carbon of glycolate:



Reaction [1] has been found localized in the peroxisomes of leaf cells (31), and these organelles contain a large excess of catalase activity. Thus, if the production of CO_2 from glyoxylate occurs in a manner similar to Reaction [2], it must occur somewhere in the cell other than the peroxisomes.

TABLE 3. Requirements for the enzymatic decarboxylation of $[1-^{14}\text{C}]$ glyoxylate by spinach chloroplasts (ref. 35)

Reaction mixture	$^{14}\text{CO}_2$ produced ($\mu\text{mol/mg}$ of chlorophyll per hr)
Complete	61.0
No Mn	3.1
Dark	14.6
N_2 atmosphere	4.8
Heated chloroplasts	7.9
No chloroplasts	8.6

The complete mixture consisted of $[1-^{14}\text{C}]$ glyoxylate (10 mM), MnCl_2 (10 mM), chloroplast suspension ($32 \mu\text{g}$ of chlorophyll), and HEPES buffer at pH 7.6 (20 mM) in a final volume of 1.0 ml; 30° ; white light ($0.14 \text{ cal cm}^{-2} \text{ min}^{-1}$, 400–700 nm).

TABLE 4. Initial rate of glycolate accumulation in tobacco, maize, and sunflower leaf disks when glycolate oxidase is inhibited by α -HPMS (ref. 26)

Exp. no.	Time in 10 mM α -HPMS (min)	Glycolate accumulation		
		Tobacco (μ mol per g fresh wt per hr)	Maize	Sunflower
1	3	50.0	4.2	61.6
	2	48.8	—	—
2	2	82.6	—	—
	2	72.6	—	—
	2	62.6	—	—
	3	—	—	70.0
3	3	—	—	90.0
	3	—	—	85.0
	3	—	—	92.5
4	3	—	13.0	—
5	6	—	7.1	—

Leaf disks 1.6 cm in diameter were cut with a sharp punch, floated on water at 30°, and illuminated with 16,000 lux in air. After 90 min, the water was withdrawn and replaced with 10 mM α -HPMS for the times shown; the leaf disks were then killed (six disks with a fresh weight of 240 mg in each sample) and analyzed for glycolate accumulation. The different values shown for some experiments were obtained from leaves excised from different plants.

Some investigators believe that glyoxylate must first be converted to glycine, and that the CO₂ in photorespiration is produced during the complex reaction by which two glycine molecules condense to produce serine, as shown in Fig. 2 (32, 33). Recent experiments comparing tissues supplied with [*1-¹⁴C*]glycolate and [*1-¹⁴C*]glycine suggest that the condensation of glycine to serine does not produce sufficient CO₂ to account for photorespiratory rates (34), and that a direct photooxidative decarboxylation of glyoxylate (Fig. 2) is more likely to be the primary source of this CO₂ (35). This view is supported by the demonstration that isolated chloroplasts can perform the rapid decarboxylation of [*1-¹⁴C*]glyoxylate to form the products shown in Reaction [2] in the presence of manganese ions, light, and oxygen (Table 3). These requirements, and the rates obtained, are consistent with the participation of such a reaction in the photorespiratory process.

Inhibition of glycolate oxidase

Bisulfite addition compounds of aldehydes are salts of α -hydroxysulfonates, and have the general structure R—CHOH—SO₃Na. Such compounds are analogs of glycolate and are effective at low concentrations as competitive inhibitors of purified glycolate oxidase (36). The most effective inhibitor of glycolate oxidase *in vivo* is α -hydroxy-2-pyridine-methanesulfonic acid (α -HPMS) (5). The uptake of ¹⁴CO₂ was not inhibited in tobacco leaf disks floated on 10 mM α -HPMS, and glycolate accumulated at an initial rate of 40 μ mol/g of fresh weight per hr at 30° during exposure to the inhibitor for up to 10 min in light (37).

Under optimal conditions in air at 30°, with 2- to 3-min treatments with α -HPMS, average initial rates of glycolate accumulation, or synthesis, were observed of 67 μ mol for illuminated tobacco and 80 μ mol/g of fresh weight per hr for sunflower (Table 4). Both of these species show rapid rates of

photorespiration, and the quantities of glycolate synthesized are sufficiently rapid to sustain photorespiration if this amount of substrate is normally oxidized. The average initial accumulation for maize leaf disks under the same conditions during the linear phase of increase was 8.1 μ mol/g of fresh weight per hr. Thus, the slow rate of photorespiration commonly observed in maize (Fig. 1) may be explained to a great extent by the much slower rate of glycolate production in this tissue.

Upon addition of α -HPMS to tobacco leaf disks under the conditions of the ¹⁴C assay for photorespiration, the ¹⁴CO₂ released during illumination is severely inhibited as the glycolate concentration builds up in the leaf, while the rate of ¹⁴CO₂ output in darkness is unaffected (Fig. 3). These results provide some of the evidence that the substrate for photorespiration, unlike dark respiration, is glycolate.

Another characteristic of an efficient photosynthetic species—such as maize—is that leaf CO₂ assimilation increases greatly between 20° and 35°, while in less efficient species—such as wheat and tobacco—photosynthesis changes little over this range of temperature (Fig. 4). These differences on the effect of temperature on net photosynthesis are attributable to differences in photorespiration in the several species.

Treatment of tobacco tissue with α -HPMS at 35° increased the ¹⁴CO₂ uptake about 3-fold in short-time experiments (Table 5) when glycolate oxidation was inhibited. The inhibitor had little effect at 25° (38). Therefore, inhibition of the production of photorespiratory CO₂ temporarily converted normal tobacco into a tissue that mimics the photosynthetic rates of the more efficient maize. This result means that sufficient biochemical acceptors for CO₂ and an adequate photochemistry are not lacking in tobacco, but that the photorespiratory production of CO₂ must largely account for the differences, as cited in Table 2 and Fig. 4, that naturally occur in net photosynthesis between these species. Gross photosynthesis must normally also increase at warmer temperatures in species like tobacco, but it is usually masked by the rapid flux of photorespiration.

To account for these results of increased photorespiration masking increased CO₂ uptake, a model balance sheet of CO₂ uptake and evolution has been prepared (Table 6). The budget (3) suggests that the elimination of photorespiration in an inefficient species may increase net photosynthesis by 47% at 25°, and by as much as 170% at 35°.

Control of photorespiration within a species

Thus far, differences in photorespiration have been discussed that result in differences in CO₂ assimilation and plant pro-

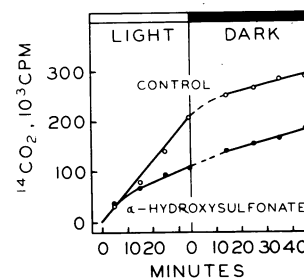


FIG. 3. ¹⁴CO₂ released by tobacco leaf disks in the presence of α -HPMS at 35°. The inhibitor affects the rate of light, but not dark, respiration. At zero time (see Fig. 1), the fluid was replaced with water or 10 mM α -HPMS. Stomatal widths were not affected by the treatment (17).

TABLE 5. Effect of α -HPMS on $^{14}\text{CO}_2$ uptake by tobacco leaf disks in light at 35° (ref. 38)

Exp. no.	Min in $^{14}\text{CO}_2$	μmol of $^{14}\text{CO}_2$ uptake per g fresh wt per hr	
		Disks in water	Disks in inhibitor
1	5	31	66
2	2.5	45	268
3	3	47	203
4	4	61	181
5	2.5	58	128
6	2.5	37	188

After leaf disks on water were kept for a preliminary period at 21,400 lux of illuminance, the fluid in Warburg vessels was replaced by 10 mM α -HPMS (5 mM in Exp. 6). After 2 min, 3 μmol of $^{14}\text{CO}_2$ (initial concentration about 0.1%) was liberated into the gas phase for the time shown.

ductivity between species. Besides attempting to learn how to control photorespiration by biochemical methods, my colleagues and I have also been experimenting with the control of photorespiration within a species that usually shows high rates by the use of pedigree selection and genetic methods. In addition to the results described below, differences in photorespiration have been found in varieties of beans with the ^{14}C assay (39) and in clones of ryegrass (*Lolium*) with an O_2 uptake assay (40).

Our first studies were concerned with a spontaneous mutation of the tobacco variety John Williams Broadleaf (JWB) possessing yellowish leaves, first described by Burk and Menser (41). The yellow mutant (JWB Mutant) is dominant (*Su/su*), and is viable only when heterozygous. When a yellow plant is self-pollinated, 25% of the progeny are white and do not survive (*Su/Su*), 50% are the yellow plants (*Su/su*), and 25% have dark-green leaves (JWB Wild or *su/su*). At high concentrations of CO_2 (0.45–5%) the mutant performs rapid photosynthesis (42), but at normal CO_2 levels it has an inferior CO_2 assimilation (43) and grows poorly. The low rate of photosynthesis and poor growth result from an unusually high rate of photorespiration in a normal environment (43).

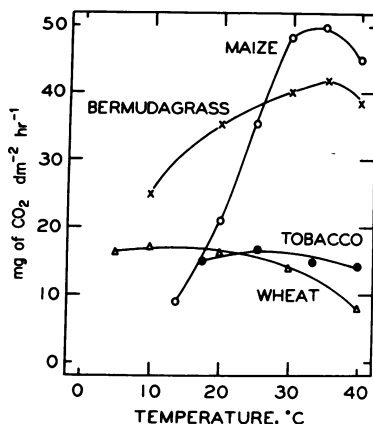


FIG. 4. Effect of temperature on net photosynthesis in single leaves of several species at 300 ppm of CO_2 in air in bright light (3).

TABLE 6. Model of CO_2 budget for a leaf with a rapid rate of photorespiration at 25° and 35° in high illuminance and 300 ppm CO_2 in air (ref. 3)

	mg of CO_2 dm^{-2} hr^{-1}	
	25°	35°
1. Gross photosynthetic CO_2 uptake	-25.0	-47.0
2. CO_2 output from dark respiration	+3.0	+6.0
3. CO_2 output from photorespiration	+7.0	+26.0
4. Net CO_2 uptake obsrved	-15.0*	-15.0*
5. When photorespiration inhibited, net CO_2 uptake observed	-22.0	-41.0

* 15.0 mg of CO_2 dm^{-2} hr^{-1} is equivalent to about 114 μmol of CO_2 per mg of chlorophyll per hr.

Table 7 summarizes results on net photosynthesis and photorespiration in the yellow mutant, its green sibling, and a standard tobacco variety, Havana Seed. Net photosynthesis was inversely related to photorespiration in the three tobacco varieties, and JWB Wild had a 24% faster rate of CO_2 uptake than did the standard variety. Moreover, the leaf growth rate of seedlings of the three varieties was directly related to their ability to fix CO_2 as illustrated in Table 7. The genetic differences between the siblings JWB Mutant and JWB Wild, besides affecting photorespiration rates, also alter the amount of chlorophyll produced and the fine structure of the chloroplasts (42), and the rate at which the stomatal pores open in light (43). Accordingly, my colleague, Dr. Peter Day, and I began a further search for individual plants that differed in photorespiration and were normal in appearance.

Progeny of the standard tobacco variety, Havana Seed, that had gone through six unselected self-pollinations were examined by the ^{14}C assay of photorespiration. We found a normal-appearing plant that consistently gave a light/dark ratio of only 1.6, although leaves of Havana Seed usually have a light/dark ratio greater than 2.5 (Table 7). This plant with a lower than usual photorespiration was self-pollinated, and 16 of the progeny were examined for photorespiration and net photosynthesis. Six of these 16 plants had below normal rates

TABLE 7. Comparison of photorespiration and net photosynthesis in excised leaves of three tobacco varieties and hybrid maize at 300 ppm CO_2 and 16,000 lux (ref. 43)

Species	Photorespiration* ($^{14}\text{CO}_2$ released light/dark)	Net photosynthesis (mg of CO_2 dm^{-2} hr^{-1})	Photo-synthesis comparison, (Havana Seed = 100)
Tobacco (Havana Seed)	3-5	13.6 \pm 0.3	100
Tobacco (JWB Wild)	0.8-2.3	16.8 \pm 0.4	124
Tobacco (JWB Mutant)	3.7-6.6	10.8 \pm 0.6	79
Maize	<0.1	26.5 \pm 0.7	195

* Photorespiration was determined by the ^{14}C assay described in the text.

TABLE 8. Variation in photorespiration and net photosynthesis in excised leaves of two tobacco plants in the third generation sampled at different times

Low-photorespiration plant, N-7-1			High-photorespiration plant, N-7-4		
Date sampled	Photorespiration ($^{14}\text{CO}_2$ released light/dark)	CO_2 uptake ($\text{mg dm}^{-2} \text{ hr}^{-1}$)	Date sampled	Photorespiration ($^{14}\text{CO}_2$ released light/dark)	CO_2 uptake ($\text{mg dm}^{-2} \text{ hr}^{-1}$)
Dec. 31	1.5	—	Dec. 31	3.2	—
Jan. 4	—	25.1	Jan. 4	—	17.0
Jan. 12	—	22.2	Jan. 12	—	15.3
Jan. 13	1.4	—	Jan. 14	2.9	18.0
Jan. 13	2.2	21.8	Jan. 14	3.3	16.7
Jan. 13	1.6	24.5	Jan. 14	5.2	18.0
Mean	1.7	Mean 23.4	Mean	3.7	Mean 17.0

Photorespiration was determined by the ^{14}C assay described in the text. CO_2 uptake was measured at high illuminance in normal air in different leaves excised on the dates shown. There was little difference in the stomatal widths between leaves of the two plants.

of photorespiration and unusually high (above $22 \text{ mg dm}^{-2} \text{ hr}^{-1}$) rates of CO_2 assimilation for tobacco. A similar proportion of efficient plants was obtained on self-pollination of low-photorespiration plants in each of the next two generations.

One of these plants in the third generation had a mean light/dark ratio of 1.7 and a net photosynthesis of $23.4 \text{ mg of CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ when sampled over a 2-week period (Table 8). This plant in the third generation was compared with another plant with a high rate of photorespiration that was derived from self-pollinating a plant with a light/dark ratio of 3.8 and a net photosynthesis of $18.8 \text{ mg of CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$. The mean CO_2 uptake in the high-photorespiration plant was 38% less than that of the low-photorespiration plant ($17.0 \text{ mg of CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) over the 2-week period.

Thus far, our work on pedigree selection of plants with high and low rates of photorespiration in tobacco indicates that plants with low photorespiration and high photosynthetic rates when self-pollinated produce many more progeny with these characteristics than does self-pollination of plants with high rates of photorespiration. Our screening methods, based on the ^{14}C assay, appear reliable, but they are cumbersome and, even with recent improvements, we are able to assay only eight plants each day. Reliable and more efficient screening methods permitting the assay of larger populations will be necessary before the genetics of photorespiration can be easily studied in a wide range of crop plants. Nevertheless, the 38% greater net photosynthesis shown in Table 8 in a normal tobacco plant with a lower than usual rate of photorespiration indicates that if this attribute could be fixed in an otherwise suitable genetic background, large increases in plant productivity would be obtained by the control of photorespiration.

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